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ON THE ORIGIN AND FUNCTION OF THE PUPAL STAGE.

By H. E. Hinton, Ph.D.

(*Department of Entomology, British Museum (Natural History).*)

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With 1 Text-figure.

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I. Introduction.

I have recently (Hinton, 1946*a*) proposed a new classification of endopterygote pupae, based chiefly on the pupal structures that have been evolved to permit the escape of the imago from its pupal cell or cocoon. In this paper I am concerned with an attempt to answer such questions as " What is a pupa ? ", " What function does it serve ? ", and " At what stage in the evolution of insects does it arise ? ". Attempts to answer these questions have been made by Deegener (1910), Berlese (1913), Poyarkoff (1914), Handlirsch (1926), Zickan (1944) and many others. I believe that the argument developed here constitutes a complete refutation of all previous theories except that of Poyarkoff (1914).

II. Is the Pupa a Larval or an Imaginal Instar ?

No one has seriously questioned the view that the Endopterygota are derived from some group or groups of Exopterygota that are now extinct. Leaving aside for the moment some Exopterygota, such as the Ephemeroptera and the males of certain Homoptera, we can say that the Endopterygota differ from the Exopterygota in possessing a pupa.

The pupal stage of the Endopterygota always occurs in ontogeny between the final larval instar and the adult. The postembryonic cycle of the Endopterygota thus differs from that of the Exopterygota in possessing an additional stage, a stage added to the postembryonic cycle of the Exopterygota and intercalated between the final larval instar and the adult. Since new developmental stages in plants and animals can arise only as the result of the modification or division of previously existing stages, we are entitled to ask what stage or instar present in the Exopterygota has been either modified or divided to produce a pupa.

If we examine a primitive pupa, such as that of the snake-fly, *Raphidia*, we are at once struck by the fact that in nearly every respect it is precisely like the adult. In external form it differs from the adult only in having thick wing-pads instead of the much larger and more delicate, multiveined wings.[1] Its head, antennae, mouth-parts, thorax, legs, and abdomen are, apart from differences in pigmentation and setae, exactly like those of the adult. In no way does this pupa resemble its larva, and we are left with the problem of why the larva is unable to change directly into the adult, since it can change directly into a stage that hardly differs in external form from the imago. So far as external morphology is concerned, the pupa of the snake-fly is in no sense a developmental stage half-way between the larva and adult. If we now turn to the pupa of one of the most highly specialized insects, such as that of a blow-fly, we again see that the pupa possesses all the important external characters of the adult apart from having shorter wings without a distinct venation. The pupa of the blow-fly in no way resembles its larva. The latter is without a distinct head capsule or the usual cephalic appendages, and it has no legs. What is obvious enough in the case of the snake-fly is even more obvious here—the pupa is not a half-way stage between the larva and the adult, but is a kind of an adult. In the case of the blow-fly it is even more difficult to see why the larva does not change at once into an adult, since in one moult it can discard all its special larval structures and acquire all the enormously different external structures of the imago. A further fact of some interest in this connection is that, except in the females of some Strepsiptera and Lycidae which have no pupal stage, only one instar intervenes between the larva and adult of all Endopterygota, regardless of how great or how little may be the difference between the larva and adult.

In many Exopterygota the final larval instar is very similar to the imago, and this has led some writers to suggest that the pupa of the Endopterygota corresponds to the final larval instar of the Exopterygota, which has become immobile. If this theory were correct, we might, perhaps, expect to find among recent Exopterygota a differentiation between the final larval instar and those preceding. For instance, there is a considerable difference in form between the larvae and adults of some aquatic Exopterygota, such as the Odonata. But the final larval instar of these resembles the penultimate instar quite as much as a locust or a cockroach final instar larva resembles its penultimate instar. Furthermore, the structural differences between the final larval instar and the adult are considerably greater in the Odonata than in the

[1] There is a lightly impressed venation on the pupal cuticle which is different from the imaginal tracheation (miscalled "pupal tracheation") that develops within the wing of the pharate adult.

BLATTIDAE and ACRIDIDAE. No positive evidence has been brought forward to support the view that the pupa has been evolved from the final larval instar of the Exopterygota, and we can find no early stages of such a development amongst recent Exopterygota. This theory of the origin of the pupa must, therefore, be abandoned for lack of evidence.

From what has been said, it appears that two important facts prevent us from regarding the pupal stage as a stage homologous to the final larval instar of the Exopterygota : (1) The external anatomy of a primitive pupa is in all essential respects similar to that of the adult, and it possesses none of the anatomical characteristics which distinguish the larva from the imago ; and (2) among recent Exopterygota which have larvae that differ greatly from their adults (e.g. Odonata) there is no evidence that the final larval instar differs more from those preceding than in groups that have larvae that closely resemble their adults. If the theory that the pupa corresponds to the final larval instar of the Exopterygota is rejected, it appears that we can only account for the origin of the pupa by assuming that the single instar of the adult stage of the Exopterygota has been divided into two instars by an imaginal moult. If this is so, the essential distinction between nearly all exopterygote and endopterygote insects is that the imaginal stage of the former comprises only a single instar, whereas that of the latter consists of two instars.

If the exopterygote ancestors of the Endopterygota were able to acquire an imaginal moult, we might expect to find some recent Exopterygota in which the ability to moult in the imaginal stage is retained. And this is indeed the case, for Wigglesworth (1940) has shown that what would appear to be a comparatively slight upset in the hormone balance will cause the bug *Rhodnius* to moult after it has become an adult. Further experiments may show that, although latent, the ability to moult in the imaginal stage is widespread in the Exopterygota.

Imaginal moults are common in other Arthropods, *e.g.* in some groups of the Crustacea and Arachnida. Such moults are also known in some Apterygota. Amongst the Pterygota, an indisputable imaginal moult occurs in all Ephemeroptera. In the Ephemeroptera or may-flies, the first imaginal instar has been given the name of " subimago." The may-flies are one of the older orders of insects, of which there is considerable fossil material, and both imaginal instars have been found in the Lower Permian rocks of Kansas.

No one has seriously disputed the fact that the so-called " subimago " of the Ephemeroptera is the first imaginal instar ; and it may be convenient to retain the term " subimago " to designate the first imaginal instar of may-flies. Similarly, even if the theory here expounded is correct, the term pupa can and no doubt will be retained for the first imaginal instar of the Endopterygota. The use of special terms, however, is apt to blind us to fundamental relationships. As soon as the terms " subimago " and " pupa " are equated to " first imaginal instar," it is difficult to avoid asking ourselves what is the difference between the first imaginal instar of the Ephemeroptera and the Endopterygota, or, in other words, what is the difference between the subimago of may-flies and the pupa of the Endopterygota. The only obvious difference that comes to mind is that the subimago is much more active than a pupa, and possesses wings with which it may fly—but I will return to this problem later.

If it is agreed that the subimago and pupa are homologous instars, it is difficult to avoid the conclusion that the subimago is a primitive kind of pupa. The subimago has remained basically unaltered since the Lower Permian, and the second imaginal instar of recent may-flies does not differ much from those of the Lower Permian, which had the fore and hind wings similar in size and venation. In the Lower Permian rocks are also found the first representatives of the evolutionary lines that gave rise to the modern orders of endopterygote insects. In this case, however, the differences between recent forms and their Lower Permian ancestors is very much greater, and we may therefore tentatively suppose that there were also great differences between Permian and recent pupae.

In the Endopterygota and Ephemeroptera there are two moults, no less and no more, between the last larval instar and the imago. The first is required to transform the insect into a pupa (1st imaginal instar), and the second is required to transform it from a pupa to an active adult (2nd imaginal instar). The problem is to discover the functional significance of the additional moult in the Endopterygota and Ephemeroptera. It has already been stated that, except for the wings, the external structure of a primitive pupa is more or less identical to that of its adult. At first sight it would appear that the two moults are required because the wings develop internally in the larvae of the Endopterygota. Against this, however, we have the fact that in the Ephemeroptera, which likewise require two moults, the wings of the final larval instar are external as in other winged Exopterygota with only one moult between larva and adult. Furthermore, if the wings can be evaginated in one moult—larva to pupa—it is hard to see why they cannot at the same time be completed and appear fully formed. It is also hard to accept the wings as the reason for two moults when we see that in the higher Diptera a complete head, mouth-parts, antennae and legs are all acquired in one moult. The invaginated external parts also require only one moult. The enormous changes that occur in the respiratory system of the Odonata between the last larval instar and the adult may be cited as an example of this.

For reasons given in the preceding paragraphs, it would appear that the significance of the extra moult in the Endopterygota must be sought for in the development of one or more of the internal organ systems. With this idea in mind I began to search through published accounts of the metamorphosis of different endopterygote insects, and I eventually chanced on a little-known paper by Poyarkoff (1914). This paper not only explains for the first time the functional significance of the pupa, but it also contains a very good summary of the principal theories that had been advanced to account for the origin of the pupal stage.

III. The Pupa and the Metamorphosis of the Skeletal Muscles.

Poyarkoff (1914) points out that in the Endopterygota the only internal organs that absolutely require two moults between the final larval instar and the adult are the skeletal muscles. Henneguy (1906 : 130) appears to have been the first to show that the muscles are attached to the cuticle by means of fibres or " tonofibrillae." These tonofibrillae thus serve as intermediaries between the muscles and the cuticle. Although the exact nature of the tono-

fibrillae is not yet clear, it is not disputed that the skeletal muscles can only secure their attachments to the cuticle at the time the cuticle is being laid down.

The adults of nearly all Endopterygota possess muscles that are not present in the larvae. This is particularly evident if we look at the muscles of a specialized insect such as a blow-fly. The blow-fly maggot has an altogether different set of muscles in its thorax, and all the large muscles which the adult uses for flying and walking are absent. The head of the maggot is also much reduced, and its muscles are few as compared with those of the adult. Before the adult can fly, walk, or eat it must acquire numerous skeletal muscles not possessed by its larva.

The skeletal muscles develop in a straight line between two points whose distance apart must be more or less exactly fixed. If these points are too near to each other the muscles would be curved. If they are too far apart the attachment of the muscles to the cuticle could not be secured. To develop the adult muscles a mould of the shape of the adult with its cuticular parts having the same spatial relations as in the adult is therefore required. This mould is the pupa. Since the secretion of the cuticle of the mould necessarily precedes the development of the muscles of the adult, the muscles cannot be attached to the mould. Once the muscles of the adult have been formed, they must be attached to the cuticle before they can function, and for this there must be another moult—pupa to adult—and a new cuticle must be secreted.

Once a pupal stage is evolved it may, in its subsequent evolution, acquire through selection new functions and structures which have no bearing on the origin of the pupal stage itself. In a broad general way it appears that the degree or amplitude of metamorphosis is greater in specialized than in primitive insects, which is but a reflexion of the fact that the adaptive divergence between larval and imaginal stages is greater in specialized than in primitive insects in respect of any organ, and also in respect of the number of organ systems involved. In individual insects a similar phenomenon may be observed proceeding from the anterior to the posterior segments. The amplitude of metamorphosis varies inversely with the rank of the segment. In the embryo the differentiation of the segments proceeds from the anterior to the posterior end, and, broadly speaking, the first or cephalic segments undergo a greater degree of specialization than the following. Similarly, the thoracic segments develop in the embryo before the abdominal, are more specialized than the abdominal in both larval and imaginal stages, differ more between larva and imago than do the abdominal, and, as a consequence, undergo a more profound metamorphosis than do the abdominal. The absence of all or all but a few pairs of primitive abdominal appendages in the larvae of the Pterygota results in a further simplification of the abdominal segments, and enhances the differences between the thorax and abdomen.

Even the most primitive of recent endopterygote pupae are highly specialized, and I know of none that are capable of moving more than the abdominal segments. In most pupae some of the skeletal muscles of the abdomen are carried over directly from the larva and are attached to the cuticle of the pupa, these muscles persisting without appreciable change in the imago. All (?) of the skeletal muscles of the head, thorax, and appendages of the larva are destroyed or undergo considerable alterations in the pupa, and none appear to be attached to the pupal cuticle. The appropriate cephalic and thoracic

muscles of the imago are formed in the pupa, and are attached to the imaginal cuticle when the latter is secreted.

It may be supposed that in pupae more primitive than any now existing in the Endopterygota only a few of the skeletal muscles (probably only some of the indirect flight muscles) were destroyed or altered, and that most of the larval muscles persisted into the second imaginal instar. Such a primitive pupa, we may further suppose, could walk about and probably lived only a very short time, since few fundamental alterations were required between larva and imago. The ancestral pupa of the Endopterygota may have been an active winged stage of very short duration, like the subimago of recent may-flies.

Poyarkoff calls attention to the paper of Dürken (1907), who showed that in the subimago (pupa or first imaginal instar) of the Ephemeroptera some of the thoracic muscles which are concerned with flying are unstriated. These muscles become striated in the imago. The striation of the skeletal muscles does not appear until they secure their attachments to the cuticle, and for this reason it may be concluded that these particular thoracic muscles of may-flies are incomplete when the subimaginal cuticle is secreted, are completed in the subimago, but are only attached to the cuticle in the imago.

If the arguments developed in the preceding paragraphs are correct, it may be said that a pupa first appears in the postembryonic cycle when the anatomical differences between the larval and adult stages become so great that muscles required by the adult cannot be formed in the larva. Once a pupal stage appears in ontogeny, a previously existing limit on the degree of divergence in form that could exist between larvae and adults is suddenly removed, and the larvae can be selected for the first time for life in environments which they previously could not occupy owing to the necessity of preserving a form approximately similar to that of the adult.

IV. The Pupa of *Pseudococcus*.

The developmental cycle of the males of certain Homoptera (Coccidae) is most instructive. The genus *Pseudococcus* may be considered as a type example. The life-history of *Pseudococcus longispinna* Targ. has been briefly described by James (1937), and from the point of view of this paper its life-history does not differ materially from that of other species of the genus. The female larva has three instars, all of which feed and are morphologically rather similar. The third or final instar larva transforms directly into the larviform female, from which it differs but little. The male larva likewise has three instars. After it has fed for a time, the second instar larva constructs a cocoon in which it transforms to a third or final larval instar, often called "pronymph" or "pseudopupa." The third instar remains in the cocoon, but it will secrete a new cocoon if it is removed from the old. It does not feed. Its front wing-pads are very distinct, and the hind wing-pads, though small, are externally visible. In the first two instars the front wing-pads are only indistinctly developed. The third instar larva transforms directly into a pupa which has a more convex body, longer antennae, and much longer wing-pads, and has a head shaped much more like that of the adult. If pulled out of its cocoon, the pupa is able to crawl slowly and can secrete another cocoon. The pupa

transforms into the adult, which is similar in shape but has well developed front wings with which it can fly.

An excellent comparative account of the skeletal musculature of the larval and imaginal instars of the males of *Pseudococcus* has been given by Mäkel (1942). Her detailed account is marred only by her failure to distinguish accurately between the different instars,[2] but carefully interpreted, her results confirm Poyarkoff's opinion of the function of the pupa in the development of the imaginal skeletal musculature. Her work, unknown to herself, not only confirms Poyarkoff's viewpoint, but it also shows that Poyarkoff was mistaken in dismissing so lightly the pupae of some Homoptera.

The total number of different skeletal muscles in the larval instars and imago of *Pseudococcus* is approximately 200, of which many are actually groups of muscles rather than single muscles. Of these, approximately 20

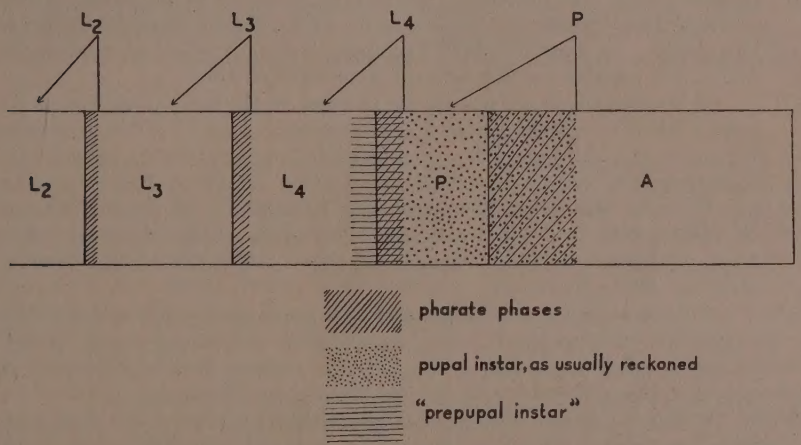

FIG. 1.

are new muscles that either arise from the larval muscle-fibre syncytium, or from scattered myoblasts that are independent of the larval muscles. For the growth of some of these, *e.g.* some of the indirect flight muscles of the thorax, a mould of the shape of the adult is indispensable.

Both sexes of the Thysanoptera and ALEURODIDAE almost certainly have a pupa in the sense that they have a stage which serves primarily as a mould in which certain imaginal muscles acquire their definitive shapes, but further

[2] Mäkel believes that the shedding of the cuticle marks the end of one instar and the beginning of another. For most but not all purposes the duration of the instars may be satisfactorily reckoned by noting the shedding of the cuticles. A new instar, however, actually begins when the old cuticle is loosened from the epidermis, and the parts of the old cuticle that have not been dissolved are only mechanically connected to the epidermis of the new instar. When the new instar is some hours, days, or even months old, the remains of the cuticle of the previous instar are shed. I have proposed (Hinton, 1946*b*) the term *pharate* to designate the phase of an instar which is enclosed within the cuticle of the previous instar (see fig. 1). Mäkel's figures have to be relabelled as follows: (5) pharate third instar, *not* late second instar; (7) and (8) pharate pupa, *not* late "pronymph" or third larval instar; and (12) and (13) pharate adult, *not* pupa shortly before the moult.

work on the ontogeny of the skeletal muscles of these groups is required. In a study of the development of the ALEURODIDAE, Weber (1934) has come to the conclusion that these are not holometabolous insects, but his evidence for this is nil, as he has not paid due attention to the skeletal muscles.

V. ONTOGENY OF THE SKELETAL MUSCLES.

The skeletal muscles of the Endopterygota and holometabolous exopterygota may be placed in three distinct groups according to their fate in ontogeny :

(1) Muscles which more or less maintain their integrity throughout post-embryonic life but undergo slight increases in length in the first or exuvial phase of each instar, *i.e.* after the epidermis is free from the cuticle of the previous instar but before it has secreted a new cuticle. Some may divide or two or more fuse together in the exuvial phases or in the pupa. In the males of *Pseudococcus* the vast majority of the skeletal muscles belong to this group. In most if not all Endopterygota only a few or none of the abdominal muscles are carried over from larva to adult.

(2) Muscles which are lost in one or other of the larval instars or in the pupa. Mäkel's work shows that in *Pseudococcus* at least 15 of 200 $\pm$ muscles belong to this category. The vast majority of the larval muscles of the Endopterygota belong here. In some highly advanced Endopterygota, *e.g. Calandra* and some MUSCIDAE and Chalcidoidea, no skeletal muscles are carried over from larva to adult. The adults of *Anthrenus* and other beetles which are unable to move in the pupal stage probably also have no larval skeletal muscles.

(3) Muscles which are completed in the pupa and are functional for the first time in the imago. In some insects some of these may begin to form in one or other of the larval instars. The muscles of this group may be placed in two subgroups according to their mode of formation :

(A) Muscles formed from myoblasts which are part of the larval muscle-fibre syncytium. These are the " Transformationsmuskelen " of Mäkel and other German writers. In *Pseudococcus* only seven of the 200 $\pm$ skeletal muscles belong to this subgroup. Most of them are the indirect flight muscles of the thorax. In some Endopterygota, *e.g. Calandra*, most of the imaginal muscles may belong to this group.

(B) Muscles formed from scattered myoblasts. In *Pseudococcus* 13 of the 200$\pm$ skeletal muscles belong to this subgroup. In *Calandra* such muscles as those of the antenna and legs of the imago belong to this subgroup. In some MUSCIDAE (*Calliphora*) and Chalcidoidea (*Momonella*) all of the imaginal muscles are of this type.

The papers of Poyarkoff (1910), Pérez (1910*a*), Tiegs (1922), Murray and Tiegs (1935), Mäkel (1942) and others enable us to estimate very roughly for several different insects the relative proportions of the total number of skeletal muscles that may be assigned to each of the muscle groups listed above. A comparison of these proportions reveals the fact that in pupae which are on other grounds regarded as primitive, a high proportion of the muscles belong to the first group and a low proportion to the second and third groups, whereas in pupae generally considered to be specialized a very high proportion of all the muscles belong to the second and third groups.

The degree to which the larval muscles are replaced by independent imaginal muscles is an indication of the degree to which the pupa is specialized ; and, in a general way, the success with which this rule may be applied varies directly with the degree of the genetic relationship of the groups being compared. In the Diptera, for instance, many of the abdominal muscles of the larva are carried over into the pupa and adult in nearly all (or all ?) Nematocera, as first shown by Weismann in *Corethra*. This is also the case in many of the Brachycera-Orthorrhapha, but in the higher Brachycera-Cyclorrhapha none of the skeletal muscles of the larva are retained in the imago. When all of the imaginal muscles belong to group "(3)," the proportion of muscles in subgroup "(B)" is greater in the more specialized insects ; and at least in some of the higher Cyclorrhapha all of the muscles belong to subgroup "(B)."

The ontogenetic sequences of the skeletal muscles of many more insects must be examined before certain other generalizations may be made. For instance, the few available data indicate the possibility that the larval muscles are lost as the pupae become specialized in fairly large groups and not one by one.

VI. What Constitutes Metamorphosis of the Internal Organs ?

The word metamorphosis simply means change of form, and if used in this loose sense it is clear that metamorphosis of at least some of the internal structures not only occurs in the development from larva to adult, but also in the successive larval instars of all insects. Handlirsch (1926) restricted the term metamorphosis to the more or less marked change of form that takes place when the insect becomes adult, irrespective of whether or not a pupal stage is intercalated between larva and adult. When the change in form is very slight, as in most Apterygota, metamorphosis is said to be very slight or wanting ; and such insects are called Ametabola. In other insects the change between larva and adult is more marked, but is accomplished in a single moult without the intervention of a pupal stage ; and such insects are called Hemimetabola, or insects with " incomplete metamorphosis." In a few exopterygote and very nearly all endopterygote insects the change between larva and adult is very marked and two[3] moults are required, a pupal stage intervening between larva and adult. Such insects are called Holometabola or insects with a " complete metamorphosis " ; and until recently certain holometabolous exopterygota were called Hemimetabola, the categories Hemimetabola and Exopterygota being used to include precisely the same taxonomic groups.

In holometabolous insects, that is insects with a " complete metamorphosis," it is evident at first sight that the amount of change of all the internal organs is not equally great, and that in not all holometabolous insects is there a " complete metamorphosis " of all internal organs. We may therefore ask ourselves what organs undergo in the pupa a change of form qualitatively different from the changes of form that occur between successive larval instars ? Or are all the changes in the pupal stage essentially similar in kind, but at most differing only in degree from those that take place between different larval instars ?

[3] The only positively known exception is the highly specialized female of the endoparasitic Strepsiptera, which requires only one moult between larva and adult.

Since the pupal stage first appears in evolution as a mould with the sole function of permitting the formation of a few muscles, probably only some of the thoracic muscles connected with flight, it might be supposed that changes qualitatively different from those that occur between larval instars are restricted to a few of the thoracic muscles. In such a pupa no other structures or tissues undergo a change that differs very much in degree from the changes undergone between one larval instar and another, or those between the final larval instar and the imago of some hemimetabolous insects. In the Ephemeroptera and some of the holometabolous Homoptera, the changes that might be considered to be qualitatively different from those between their larval instars appear to be confined to the skeletal muscles. Weber (1934) has shown that in the ALEURODIDAE, apart from the skeletal muscles, to which he did not pay much attention, no " metamorphosis of the holometabolous type " is undergone by the internal organs.

As compared with such primitive pupae, those of the Endopterygota are much more highly specialized, and a much higher proportion of the skeletal muscles undergoes a change qualitatively different from that which occurs between different larval instars. Furthermore, endopterygote pupae are more or less quiescent, have to a large extent ruptured relations with many factors of their external environment, and have a relatively long life. For all these reasons there is an opportunity for very great changes to take place in the internal organs, changes in no way connected with the acquisition of a pupal stage, and for which such a stage is not absolutely required. On general theoretical grounds it is therefore to be expected that in primitive pupae, apart from the skeletal muscles, none of the internal organs (as distinct from the invaginated external parts) would undergo changes essentially different from those between larval instars, but that some organs may do so in highly specialized pupae; and from accounts of the metamorphosis of various insects it is clear that this is the case. From a phylogenetic point of view there is nothing remarkable or unexpected in the fact that even in highly specialized pupae many of the internal organs do not undergo a change in any way different from that which takes place between one larval instar and another.

The changes which are undergone in the postembryonic development of internal organs are perhaps best known for the mid-gut. A summary of the changes undergone by the mid-gut of various insects has recently been given by Henson (1946).

In some insects, *e.g. Galleria* (PYRALIDAE), the epithelium of the mid-gut is renewed at each larval instar from replacement cells, *i.e.* embryonic cells that are activated at the beginning of each instar. In the pupa a similar renovation occurs, and the pupa has its own special epithelium. The pupal epithelium is subsequently replaced in a similar manner by the adult epithelium. In these insects, then, there is no question of a change occurring in the pupa that is qualitatively different from that which takes place in each larval instar, and there is therefore no question of a metamorphosis of the mid-gut in the sense in which that word is sometimes used. In some of the more specialized Lepidoptera, *e.g. Aglais* (NYMPHALIDAE), the renovation of the mid-gut epithelium is partly suppressed in the larval instars and is therefore incomplete. A complete renovation occurs in the pupa, and the renovated mid-gut is believed to persist in the imago as is the case in *Hyponomeuta*, *Malacosoma* and

Bombyx. The changes in the pupal mid-gut thus differ only in degree from those occurring in the larval instars. In *Calliphora* no larval renovation occurs, but renovation is total in the pupa. In view of the more or less total renovation that occurs in the larval instars of some Endopterygota, we cannot consider that the renovation of the pupal mid-gut of *Calliphora* is a process peculiar to the pupal stage.

In many beetles renovation of the pupal or both the pupal and imaginal mid-gut consists of an activation of embryonic replacement cells, and is similar to the kind of renovation that often occurs in the larval instars. However, in some beetles, *e.g.* CUCUJIDAE, NITIDULIDAE, COCCINELLIDAE, CERAMBYCIDAE, CHRYSOMELIDAE, CURCULIONIDAE and SCOLYTIDAE (Mansour, 1934), the larval mid-gut may be completely destroyed and a new pupal mid-gut arises from the so-called imaginal ring, which may be regarded as a reactivated blastoporic region (Henson, 1946). The pupal mid-gut, reformed *de novo*, as in the embryo, is in some beetles renovated in the usual way in the imago; and these morphogenic processes in the pupa may be likened to the morphogenic processes that take place in the embryo, as done by Poyarkoff and Henson. The formation of new imaginal muscles in the pupa from the larval muscle-fibre syncytium or from scattered cells may also be likened to embryonic morphogenesis. But the metamorphosis of the mid-gut and other internal organs differs in an important way from the metamorphosis of the skeletal muscles; the metamorphosis of the former requires only a single moult, that of the skeletal muscles two moults between larva and adult if it is to be biologically effective.

VII. Was a Pupal Stage Evolved Before a Larval Stage with Internal Partly Suppressed Wings?

Most writers have firmly believed that the first appearance of a pupal stage in the evolution of the higher insects is bound up with the invagination and partial suppression of the larval wings, and therefore the question of which came first, pupa or larva with internal wings, never presented itself to them as a problem. Since the function of the pupal stage is to permit the development of a larval form in which the completion of certain imaginal muscles cannot take place, it is possible that the pupal stage did not appear simultaneously with the invagination and partial suppression of the larval wings. The occurrence of the pupal stage in both may-flies and the males of some Homoptera is evidence that a pupal stage may arise before the larval wings begin to develop internally, and also seems to show that these two specialized features of higher insects are not necessarily connected. It appears to me probable that the first considerable specializations in larval form that led to the appearance of a pupal stage occurred before the larvae became further specialized for internal feeding in environments in which there is a heavy selective pressure against possessing external larval wings.

It seems clear that the possession of a pupal stage is of great advantage to the Endopterygota, and has enabled them successfully to invade many of the environmental niches from which the Exopterygota are still excluded. It is well known that the pupal stage is one of the most critical in the life of the Endopterygota, and that from one cause or another the mortality in this stage

is very high. The preservation of such a stage in the life-cycle is evidence that its compensating advantages must be enormous.

The possession of a pupal stage is by no means the only factor that accounts for the greater success of the Endopterygota. The internal development and much greater suppression of the wings in the larval stages of the Endopterygota confers on them an advantage over the Exopterygota of considerable significance. The absence of external wings in the larval stages of the Endopterygota enables them to burrow in solids in the earth and in plants. An active life in the ground or in plant or animal tissues is difficult for larvae with external wings for a very simple mechanical reason. Larvae with external wings cannot move backwards in a close-fitting burrow without catching their wings on projections from the walls of the burrow. In moving backwards through earth or plant tissues the external wing pads must act like anchors, and with few exceptions the Exopterygota have failed to occupy such habitats. Some notable exceptions may be mentioned, each of which has overcome this difficulty in a special way.

The stages of ants and termites which burrow about actively in earth and wood are apterous. The movements in the soil or wood of the winged sexual stages are limited, and when new colonies are started by these the wings are shed in the case of the termites and generally bitten off in the case of the ants. In the cicadas the larval wings may be partly folded on a transverse axis. The membranous suture which permits this in the larva is present in the pharate adult, but at some time, presumably shortly after emergence, a hardening of the membrane takes place and the transverse suture is only indicated by a transverse sclerotized line. The transverse line present in the adult has given rise to some theories, most of which seek to show its significance in flying. The transverse suture is obviously functional in the larva, and it is present on the adult wing probably because in the pharate adult stage the cicada moves through the soil, and the bending of the wings of the final larval instar necessitates the bending of the wings of the enclosed adult. The possibility of bending the wings when they encounter an obstacle enables cicadas to move backwards through the soil. This theory of the transverse line on the imaginal wing of cicadas is the only one which takes adequate account of the much more developed suture present in the larva.

VIII. Secondary Loss of the Pupal Stage.

If the chief function of the pupal stage is that of a mould to enable some of the muscles of the imago to develop, it might be expected that when, through a series of adaptive modifications, the imago secondarily acquires the form of its larva it should in time lose its pupal stage. Instances of this may be observed in some Strepsiptera and beetles. The females of the parasitic Strepsiptera, which never leave their hosts, have become larviform. The shape of their bodies hardly differs from that of their larvae, and they do not go through a pupal stage as do the differently formed free-living adult males of the same species. The larviform adult females of some Lycidae apparently also lose their pupal stage, but a pupal stage is retained so long as the female differs in form from its larva.

IX. Ancestral Characters of Pupae.

If the ancestral pupa of the Endopterygota was a free-living active stage like the pupa of recent Ephemeroptera, it might possibly be expected that not every one of its structural features has become unrecognizably modified in its highly specialized modern descendants.

I have previously shown (Hinton, 1946c) that when a structure, such as the maxillary palp in some Lepidoptera, undergoes a gradual reduction in size in the imago and finally disappears, its reduction and final disappearance are appreciably delayed in the pupa. This difference between pupa and imago in the rate of loss of certain organs makes it possible to form an opinion as to the relative antiquity of the complete loss of the organs in the imagines of related groups. In *Nesosisyphus* Vinson (Scarabaeidae), for instance, the adults have no horns on the pronotum, but in the pupa there are two very long and apparently functionless pronotal horns. In related groups such horns are frequently found in the imago. The retention of such horns in the pupa of *Nesosisyphus* is evidence that (1) *Nesosisyphus* is descended from ancestors which possessed such horns on the pronotum of the imago; and (2) the loss of pronotal horns in the imago of *Nesosisyphus* must be relatively recent, since they are still so very well developed in the pupa. Other instances of similar reductions occur in the literature. A well-known case is that of the Chironomid, *Belgica antarctica* Jacobs, which has a nearly apterous imago, but a pupa with much larger wing-pads (Keilin, 1912).

I have already drawn attention to the lightly impressed vein-like markings on the pupal wings of *Raphidia*, and it is just possible that these markings represent all that is left of the venation of an ancestor with well-developed wings like the recent active pupae of may-flies. It is known that there is a renovation of the mid-gut epithelium in the pupae of the Endopterygota. In many the mid-gut epithelium is renovated again in the imago, so that the pupa has a mid-gut epithelium which is special to itself. The persistence of a special pupal epithelium serving no apparent function in endopterygote insects may be a structure which has persisted from the time when the pupa was an active feeding stage. In other words, the special mid-gut epithelium persists in the pupa after it has lost its function, as do well developed wing pads in some species which have more or less apterous adults. In most of the more specialized members of the Coleoptera, Lepidoptera and Diptera, the pupal renovation of the mid-gut is carried over into the imago, and these pupae have therefore lost another of their ancestral characters in the sense that they no longer have a special mid-gut epithelium different from that of the imago.

X. Homology of Imaginal Instars in Hemi- and Holometabolous Insects.

In a previous paragraph it was stated that the pupa was a stage added to the developmental cycle of hemimetabolous insects and intercalated between the last larval instar and the adult. That the pupa is a stage intercalated between the last larval instar and the adult is so in the sense that the second imaginal instar of holometabolous insects is functionally equivalent to the single imaginal instar of hemimetabolous insects. From another point of view it might be considered that the first imaginal instar of the Endopterygota,

Ephemeroptera and certain Homoptera is the instar that corresponds to the imago of hemimetabolous insects, the second imaginal instar being in reality the new instar that has been added to the hemimetabolous post-embryonic cycle, and that has taken over the normal functions of the imago. But any attempt to establish such homologies overlooks the impossibility of equating A with C when A plus B result from a division of C. For the same reason, when one insect has four larval instars and another five we cannot homologize the fourth of one with the fourth of the other, although from a functional point of view we might in some cases speak of them as equivalent instars.

I have made no attempt to define the terms adult or imago, but none will quarrel with the use of these terms as usually employed for the final stage of hemi- and holometabolous insects. The view might be advanced that the pupa is a kind of larval instar, and that the larval stages of animals arose in the first instance as specializations of adult stages, or, in other words, there were adults before there were larvae, as pointed out long ago by Fritz Müller. So far as the theory advanced in this paper is concerned, the more specialized quiescent pupa may be called a larva providing only that it is recognized that such a larva arose as the result of a specialization of one of the imaginal instars *after* the differentiation of one series of larval instars from the imago was already greatly advanced. Those who hold such a view of the pupa must then recognize the fact that there are two series of larval forms in holometabolous insects, one of relatively ancient origin and the other of relatively recent origin.

XI. Holometabola or Endopterygota.

Pterygote insects are placed in two main groups which are usually called Hemimetabola or Exopterygota and Holometabola or Endopterygota. The metamorphosis of the Ephemeroptera and some Homoptera is of a truly holometabolous type, and the term Holometabola is thus not exclusively applicable to the higher insects or Endopterygota. The name Endopterygota is therefore preferable to Holometabola for the higher insects which have no external larval wings and are almost certainly a monophyletic group.

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